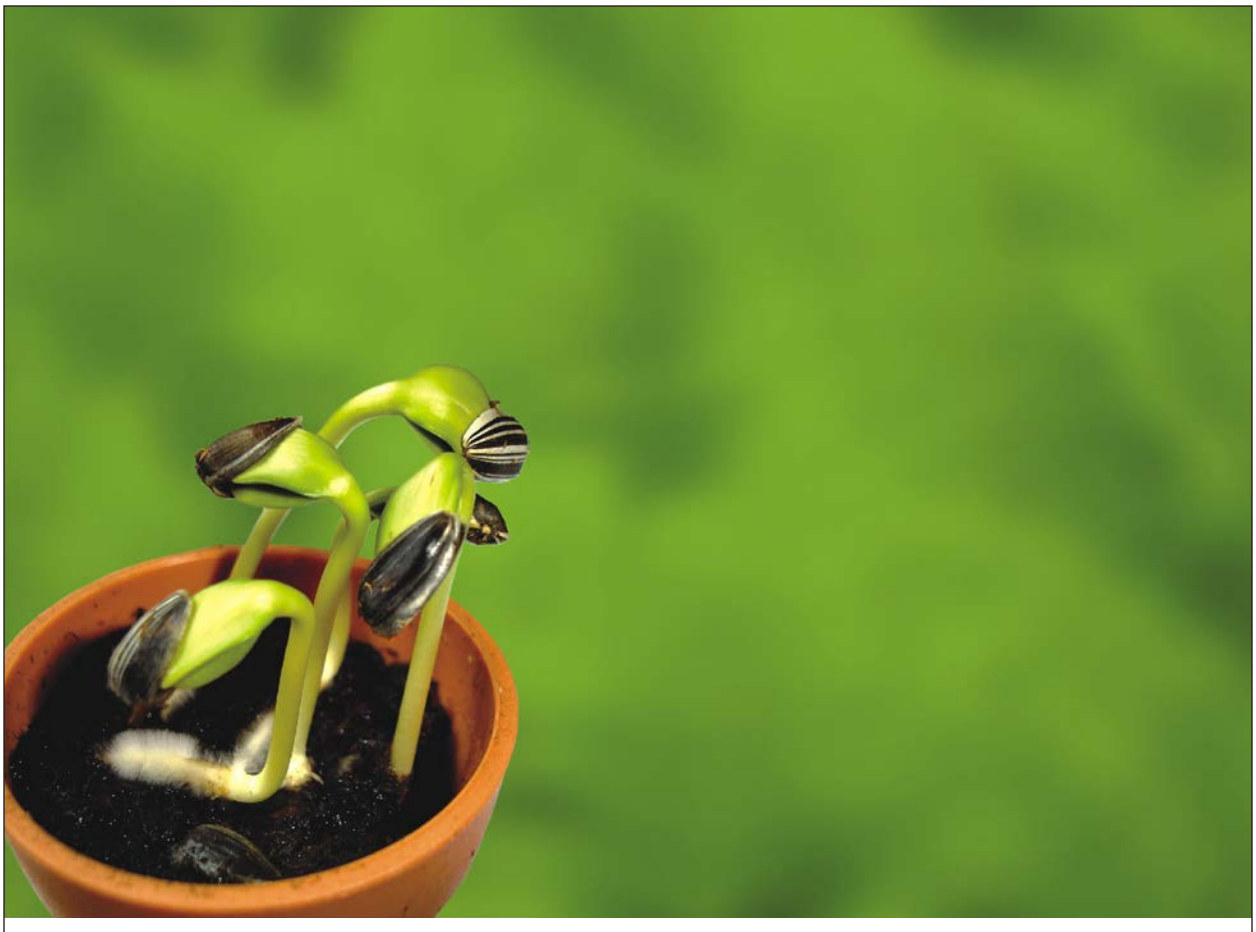

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Ascochyta blight (*Ascochyta rabiei* (Pass.) Lab.) of Chickpea (*Cicer arietinum* L.): Breeding Strategies for Resistance

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ABSTRACT

In the Mediterranean region, chickpea yield could be increased by shifting the sowing date from spring to winter. Nevertheless, this is hampered by the sensitivity of the crop to low temperatures and the fungal disease Ascochyta blight. Ascochyta blight, caused by *Ascochyta rabiei* (Pass.) Labr., is a devastating disease of chickpea (*Cicer arietinum* L.) in most of the chickpea-producing countries, including Iran. Severe epidemics of ascochyta blight have occurred many times in various production regions, often on cultivars previously thought to be resistant. The evolution of a new race or virulence form is frequently invoked to explain such outbreaks. Current cultivars only possess partial resistance to the pathogen and this level of resistance can breakdown easily because the pathogen is highly variable due to potential for sexual recombination. Although, the evaluation of the world collection of chickpea germplasm for resistance to ascochyta blight revealed a very low frequency of resistant lines, there are some resistant genotypes in chickpea germplasm which can be used in breeding programs. Marker Assisted Selection (MAS) would allow a better targeting of the desired genes. Genetic mapping in chickpea, for a long time hampered by the little variability in chickpea's genome, is today facilitated by highly polymorphic, co-dominant microsatellite based markers. More durable resistance could probably be achieved by pyramiding of resistance genes via MAS and is a major challenge for chickpea breeders. Genotypic variation has been reported for ascochyta blight resistance in chickpea using both Mendelian and Quantitative Trait Loci (QTL) analyses, with conflicting reports about the mechanism of resistance. The genetics of resistance to ascochyta blight has been extensively analysed because the disease is of great agronomic and economic importance. In this review, we summarize current situations and future prospect of necessities for changing from spring to winter sowing of chickpea as well as progresses in genome mapping and QTL analysis for ascochyta blight resistance in chickpea.

Key words: Disease resistance, chickpea breeding, ascochyta blight, QTL mapping

INTRODUCTION

In the Mediterranean region, chickpea is traditionally sown in spring and, as a consequence of the low rainfall during the growth period in dry summers, these results in poor biomass

development. Work on cold tolerance in chickpea has been initiated since, the advantages of fall-sown crop over traditional spring sown crop were realized (Singh *et al*., 1997). Winter sowing expands the vegetative growth period and improves the seed yield up to 2 t ha^{-1} (Singh and Reddy, 1996; Singh *et al*., 1995), but is rarely adopted by the farmers because the cool and wet weather, typical for Mediterranean winters, favors the development of fungal diseases. The two most important diseases of chickpea are ascochyta blight and fusarium wilt. Both of these diseases have been identified in Iran, but Ascochyta Blight (AB) is the disease of major concern. AB, caused by the necrotrophic fungus *Ascochyta rabiei* (Pass.) Lab., affects all aerial parts of the plant. Sources of resistance have been identified (Singh and Reddy, 1983) and the development of stable blight resistant lines would allow a shift to sowing into the rainy season. Since, this is currently not the case, winter planting is yet illusory in Iran (Kanouni *et al*., 2009).

To date, several sources of resistance, identified within the cultivated chickpea germplasm, have been used in genetic and breeding studies (Flandez-Galves *et al*., 2003b; Reddy and Singh, 1993; Santra *et al*., 2000; Singh, 1997; Tekeoglu *et al*., 2000). Marker Assisted Selection (MAS) can be applied effectively using DNA markers linked to resistance genes for accelerating disease resistance breeding programs.

Advances in molecular marker technology have accelerated the progress of genome mapping in chickpea. Linkage maps have been developed based on interspecific crosses between selected chickpea cultivars and *C. reticulatum* accessions (Kazan *et al*., 1993; Simon and Muehlbauer, 1997; Winter *et al*., 1999, 2000). However, most of these novel sources are unable to be readily incorporated into breeding programs because of incompatibility. In another study for identifying molecular markers for AB resistance, recombinant inbred lines from an interspecific cross between *C. arietinum* and *C. reticulatum* were subjected to marker analysis and molecular markers linked to two major QTLs, QTL1 and QTL2 were identified which together accounted for 50.3% of the estimated phenotypic variation (Santra *et al*., 2000).

The overall aim of this review is to provide better understanding of the inheritance of AB resistance. This review will also seek to document the progressive developments in AB researches and an update on progress in the development and application of molecular breeding approaches to the improvement of chickpea's resistance to AB.

Chickpea: Chickpea is an important food legume crop in the CWANA (Central, West Asia and North Africa) region, accounting for 29% of the total food legume production. Chickpea was among the first grain crops to be cultivated; dating back to the eighth millennium BC (Zohary and Hopf, 2000). It is the only widely cultivated species of the genus *Cicer* (Kupicha, 1977). The crop is a self-pollinated diploid (2n = 2x = 16) with a relatively small genome size of 740 Mbp (Arumuganathan and Earle, 1991). Chickpea is one of the first domesticated grain legume crops of the old world (Van der Maesen, 1972). The centre of origin for chickpea is Turkey and Syria (Singh, 1997), but it developed as a post-rainy season, spring-sown crop early in its evolution and spread into subtropical regions, in contrast to its wild relatives that have remained as winter annuals in West and Central Asia (Berger *et al*., 2005). Ladizinsky and Adler (1976) regarded *C. reticulatum* as the wild progenitor of chickpea based on cytogenetical and seed protein analysis and consequently nominated Southeastern Turkey as its centre of origin. This claim was supported by Van der Maesen and Pundir (1987) based on the presence of the closely related annual species, *C. reticulatum* and *C. echinospermum* in southeastern Turkey (Fig. 1).

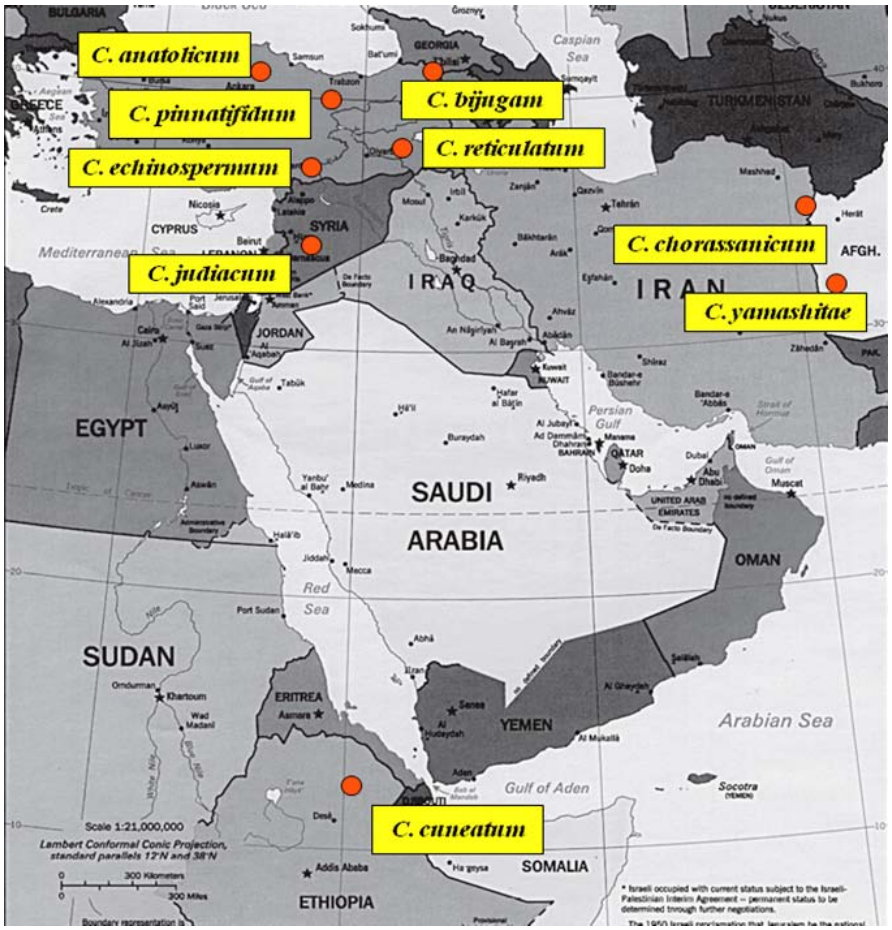

Fig. 1: Geographical locations of the wild annual *Cicer* species based on maximum genetic variation analysis

The seed is a major source of protein and energy in some regions of the world (Ladizinsky, 1995), with its seeds containing 20-30% crude protein, about 40% carbohydrate and 3-6% oil (Gil *et al*., 1996). It is a good source of calcium, magnesium, potassium, phosphorus, iron, zinc and manganese (Ibrikci *et al*., 2003). It is also used as fodder for cattle.

The optimum conditions for growth have been suggested to be 18-26 and 21-29°C, day and night temperatures respectively and an annual rainfall of 600-1000 mm (Smithson *et al*., 1985). However, Soltani *et al*. (2006) using quantitative data from four cultivars grown over three years to evaluate various approaches to predict chickpea phenology concluded that the differences among cultivars for cardinal temperatures and critical photoperiod were small. Production of chickpea contributed to agricultural sustainability through N_2-fixation and by being a rotation crop. The area harvested for chickpea in 2005 was 11,200,000 ha worldwide (Food and Agricultural Organization of the United Nations, 2005) with production of 9,172,000 T. India is the major producer of chickpea, accounting for approximately 65% of the annual world production. India is also the largest importer of chickpea. Turkey is the largest exporter of chickpea followed by Australia. The average yield of chickpea worldwide is 818 kg ha^{-1}. During 2006, Iran was the

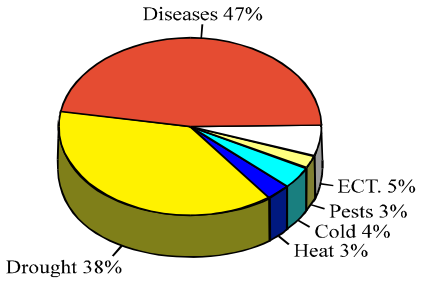

Fig. 2: Relative importance of various biotic and abiotic stresses resistance in chickpea

fourth largest chickpea producing country after India, Pakistan and Turkey (FAOSTAT, 2007). The total cultivated area, production and yield of chickpea in Iran is 735,000 ha, 288,000 t and 390 kg ha^{-1}, respectively.

Commercially, the species is grouped into desi and kabuli types: desi chickpeas generally have small, colored seeds, whereas Kabuli is produce large, cream colored ones. Furthermore, chickpea is an additional benefit to the farmers as it fixes a substantial amount of nitrogen for the subsequent crops and adds much needed organic matter that improves soil health, long-term fertility and sustainability of the ecosystems (Ahmad *et al*., 2005).

Singh (1993) proposed that under optimum growing conditions, the yield potential of chickpea is 6 t ha^{-1}, which is very high compared to the global yield level of ~0.8 t ha^{-1} (Ahmad *et al*., 2005). Berger and Turner (2007) compiled a list of biotic and abiotic stresses faced by chickpea in coarse agro-climatic zones. The chief constraints to chickpea production are biotic stresses such as Ascochyta blight (*Ascochyta rabiei*), Fusarium wilt (*Fusarium oxysporum* f. sp. *ciceri*) and pod borer (*Helicoverpa armigera*) and abiotic stresses such as drought, heat, cold and salinity (Ryan, 1997; Millan *et al*., 2006). The relative importance of research programs in alleviating different biotic and abiotic stresses of chickpea is shown in Fig. 2 (Singh *et al*., 1993). In fact, the collective yield losses due to abiotic stresses (6.4 million tons) are somewhat higher than due to biotic stresses (4.8 million tons), as estimated by Ryan (1997). Among the abiotic stresses, drought is almost ubiquitous to major chickpea growing regions and is exacerbated by heat stress in warmer Mediterranean and summer dominant rainfall areas (Berger and Turner, 2007). The frequency of occurrence and severity of these stresses varies dependant on the climatic and geographical conditions. Nene (1984) reported that there were about 41 pathogens infesting chickpea, which included 33 fungi, 7 viruses and 1 bacterium. The most important biotic stress limiting chickpea production worldwide is AB. Under favorable conditions, which are mostly cool and cloudy with higher humidity, the epidemic of AB may lead to complete yield loss (Nene, 1984; Chongo *et al*., 2004).

Ascochyta blight: *Ascochyta rabiei* is pathogenic mainly on *Cicer arietinum* and other species of *Cicer* (Millan *et al*., 2003). The pathogen is heterothallic and requires two compatible mating types, MAT-1 and MAT-2, for production of the teleomorph (sexual stage) (Kaiser, 1997; Taleei *et al*., 2008). The presence of two mating types ensures sexual recombination and leads to genetic diversity. The anamorph (asexual stage) is characterized by the presence of dark brown, spherical to pear-shaped pycnidia. The teleomorph (sexual stage) of *A. rabiei* (*Didymella rabiei* (Kovachevski) v. Arx) was first reported by Kovachevski (1936) in Bulgaria. It was later reported in Russia, Greece, Hungary, Spain, Syria and the United States (Wilson and Kaiser, 1995) and

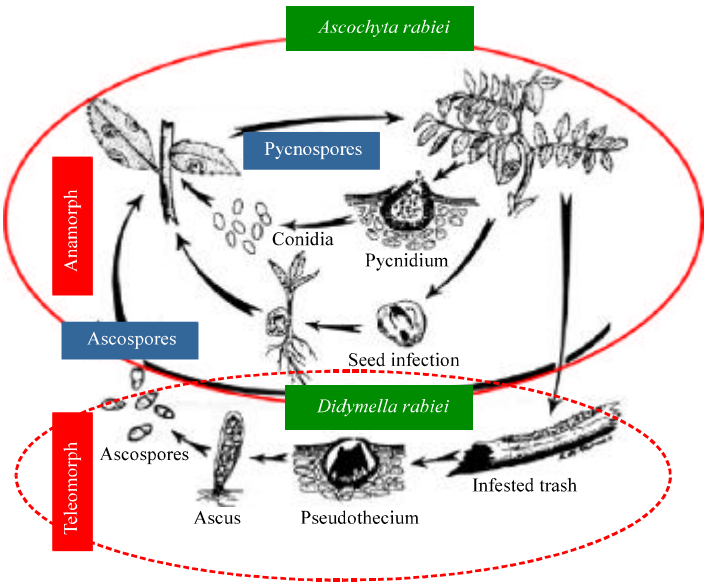

Fig. 3: Life cycle of ascochyta blight of chickpea. Both pycnidia and pseduthecia can develop on overwintered infested chickpea debris

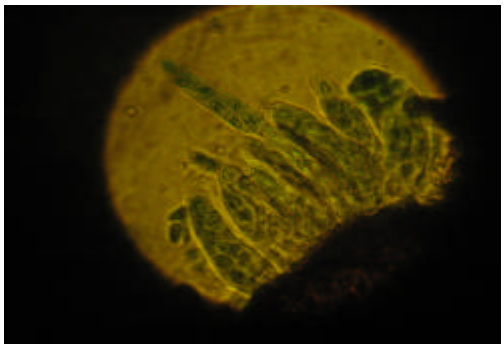

Fig. 4: Matured pseudothecia and perfect stage of ascospores

Iran (Kaiser and Okhovat, 1996). The teleomorph is characterized by dark brown to black pseudothecia on over-wintering chickpea debris. The asci are cylindrical, clavate and slightly curved. The significance of the anamorphic and telomorphic stages in the disease cycle of ascochyta blight in chickpea is illustrated in Fig. 3.

After coming into contact with host tissue, conidia of *A. rabiei* begin to germinate after 12 h (Pandey *et al.*, 1987). Penetration into the host tissue normally occurs 24 h after inoculation, through the leaf cuticle, stem cuticle and through stomatal openings (Pandey *et al.*, 1987). *Ascochyta rabiei* form typical appressoria associated with stomatal penetration (Illarslan and Dolar, 2002). Four days after inoculation, necrosis become visible and the hyphae in the cortical tissues fuse together to form aggregates. The pycnidia are found largely in the vascular tissues, possibly due to the vascular tissue providing a suitable matrix for pycnidial development when the surrounding tissues are destroyed (Kohler *et al.*, 1995). Five or six days after inoculation, the pycnidia mature, arranged in a circular pattern on the infected host tissue (Fig. 4). By the seventh day, most of the non-lignified cells are destroyed (Pandey *et al.*, 1987; Illarslan and Dolar, 2002).

The pathotype of an *A. rabiei* isolate is determined by assaying its pathogenicity on a set of deferential chickpea cultivars. Using such an assay, Vir and Grewal (1974) reported finding more than ten pathotypes among field isolates from India. In similar studies Reddy and Kabbabeh (1985) identified six pathogenic groups among 50 isolates from Syria. Nene and Reddy (1987) found five pathogenic groups and several strains, respectively, among isolates from Pakistan and Turkey. However, these workers used different assay procedures and different cultivars for the pathogenicity tests. These assays were conducted by artificial inoculation either in the field or under plastic-house conditions. In these assay procedures there was very little control on the environment, therefore the assay results necessarily fluctuate. Furthermore, Porta-Puglia *et al.* (1996) judged that pathogenicity or aggressiveness within the same isolate/line combination could vary according to the environmental conditions. For example, Reddy and Kabbabeh (1985) reported the aggressiveness of the six isolates to be in the order 6'5'4'3'2'1 under plastic-house conditions. For the same isolates Weising *et al.* (1991) reported a different aggressiveness (6'4'2'1'5'3) under growth-chamber conditions.

Classification of pathotypes of *A. rabiei* is currently based on testing isolates against a set of host genotypes with different resistance reaction to the aggressiveness of the pathogen. Udupa *et al.* (1998) classified 53 Syrian isolates into three pathotypes: pathotype I (least aggressive), pathotype II (aggressive) and pathotype III (most aggressive). Jamil *et al.* (2000) also classified 130 isolates into three pathotypes. Similarly, Shokouhifar *et al.* (2001) collected 26 isolates of *A. rabiei* from 16 provinces of Iran and clustered them into 22 genomic groups using RAPD. Chongo *et al.* (2004) have classified 40 Canadian isolates into 14 pathotypes, on the basis of their interactions with eight chickpea differentials, but other work indicates that this variability can be summarized into as few as two broad pathotypes: pathotype I (less aggressive) and pathotype II (aggressive) (Chen *et al.*, 2004). Bayaa *et al.* (2004) collected an isolate from Kaljebrine region in the North of Aleppo, Syria and called it as new pathogenic variant of *A. rabiei*, which was virulent to ICC 12004 and ICC 3996 (highly resistant genotypes).

Ascochyta rabiei infection may arise from seed-borne inoculum, conidia produced on infected debris or air-borne ascospores. AB symptoms occur on all above-ground plant parts at any growth stage of the crop, producing necrotic lesions that may result in the destruction of the plant (Nene, 1984; Shtienberg *et al.*, 2000). The symptom on lower leaves start as pin-head spots, which are usually dark tan to black in color. These spots develop into water-soaked lesions. The centre of each lesion contains the small black fruiting bodies (pycnidia) arranged in concentric circles. On stems and petioles, the lesions expand and girdle. Girdled stems break and the foliage above the break point dies. On pods, lesions lead to seeds shriveling and discoloration of the seed (Fig. 5a-c).

In the field, blight appears as small circular patches of dead plants. However, if the source of inoculum is seed-borne, disease symptoms are often scattered uniformly across the field. On resistant cultivars, although, the lesions appear as small dark brown spots, they may not progress further (Chongo and Gossen, 2003). Under moist conditions, mature pycnidia swell and the conidia ooze out. Conidia are dispersed to neighboring plants through rain splashing (Armstrong *et al.*, 2001). Under cool moist conditions, the disease spreads rapidly through the field.

Environmental conditions have important effect on the lifecycle of *A. rabiei*, the infection process and disease development. The optimum temperature for infection and development of *A. rabiei* is 20°C (Trapero-Casas and Kaiser, 1992). Asci and ascospores only develop at temperatures 5 to 10°C. Low temperature and a relatively long incubation period are required for sexual reproduction in most Ascomycetes. The lower and upper temperature limits for infection by

Fig. 5: Ascochyta blight lesions on chickpea. (a) early ascochyta symptoms on leaves of chickpea, (b) stem lesion and breakage due to girdling, (c) lesions with concentric rings of pycnidia on pods and (d) late symptoms of ascochyta blight on chickpea. Note gray centers with black margins

A. rabiei are 5 and 30°C, respectively (Trapero-Casas and Kaiser, 1992). Disease severity increased with increasing temperatures to a maximum of 20°C, then declined sharply at temperatures above 25-30°C. At temperatures above 25°C spore production and mycelial growth decrease and cease at 32°C.

Physiological mechanisms of resistance to ascochyta blight: Several factors that contribute to resistance against *A. rabiei* in chickpea have been identified; these include preformed structural and chemical components. Induced components of resistance in chickpea are hydrogen-peroxide-mediated cell wall cross-linking, production of PR proteins (chitinase, β-1,3-glucanase and thaumatin-like proteins), phytoalexin accumulation and detoxification mediated by glutathione *S*-transferase (Cho and Muehlbauer, 2004; Jayakumar *et al*., 2005). Studies to identify components of resistance are generally restricted to a few chickpea genotypes and concentrate on one component or mechanism at a time. It would be interesting to evaluate levels of these components in larger samples of germplasm. Also, the question arises whether superior resistance in certain genotypes is due to elevated expression of one, or a combination, of these resistance factors (Jayakumar *et al*., 2005). Such studies could help to identify genotypes with exceptionally high expression levels of a combination of different resistance factors or single factors.

There is a high level of variation in aggressiveness within *A. rabiei* populations (Chen *et al*., 2004; Chongo *et al*., 2004) and also a similar level of variation in resistance among chickpea cultivars (Cho *et al*., 2004; Udupa and Baum, 2003). Aggressive isolates of *A. rabiei* severely affect some chickpea cultivars that are resistant to less aggressive isolates under the same conditions.

Moreover, resistance declines when the plants reach the flowering and podding stage (Singh and Reddy, 1993; Chongo and Gossen, 2003). The symptoms of infection and disease severity follow a quantitative continuum based on aggressiveness of the pathogen, genetic resistance present in the cultivar and age of the plant.

Investigations of resistance mechanisms have revealed that growth of *A. rabiei* stops in the apoplastic space, whereas hyphae rapidly spread in the apoplastic space in susceptible cultivars. The apoplastic space is the interface for plant-pathogen interactions and contains plant and pathogen gene products that are responsible for specificity of the disease interaction. The lignified tissues and pith parenchyma of resistant cultivars are not attacked by the pathogen (Angelini *et al*., 1993; Illarslan and Dolar, 2002). In general, the infection process is delayed in resistant cultivars and the area of fungal colonization and pycnidium formation is restricted. Necrotic flecks produced during the initial stages of disease development, as well as restriction of the growth of *A. rabiei* in the apoplastic space, both indicate that defense mechanisms in resistant chickpea cultivars are activated soon after infection. It is important to note, however, that complete resistance to *A. rabiei* has not been found in chickpea and that the resistance present in superior cultivars is partial or incomplete.

Immunochemical studies on resistant and susceptible cultivars have indicated that polyamine metabolism and subsequent release of hydrogen peroxide (H_2O_2) in the apoplast contribute to resistance in chickpea plants inoculated with *A. rabiei*. Several classes of enzymes produce H_2O_2 in the apoplast: plasma-membrane NADPH oxidases, cell-wall oxalate oxidases, peroxidases (PODs) and flavinand copper-containing amine oxidases (CuAOs) (Rea *et al*., 2002).

Pathogenesis-Related (PR) proteins are defined as plant proteins that are induced during infection by the pathogen or wounding. Pathogenesis-related protein families are grouped into at least 14 classes, primarily on the basis of their amino acid sequence (Van Loon and Van Strien, 1999). Although, the biological and biochemical function of many PR proteins remains unknown, several possess antimicrobial properties. Attention has focused on fungal chitinases and **β**-1, 3- glucanases because of their hydrolytic activity on cell walls of invading fungal pathogens. Chitinases act alone or in synergy with **β**-1, 3-glucanases in degrading fungal cell walls and inhibiting fungal growth (Jayakumar *et al*., 2005).

Genetics of resistance to ascochyta blight: Evaluation of chickpea germplasm has shown that there are very few accessions with resistance to AB (Reddy and Singh, 1984, 1992). However, wild species of *Cicer* such as *C. echinospermum* have some resistance (Collard *et al*., 2001). Both *C. reticulatum* and *C. echinospermum* are cross-compatible with *C. arietinum* and could provide sources of resistance (Singh and Ocampo, 1993).

Interspecific hybridization: Wild relatives that are closely related to the cultivated chickpea, have the potential to provide the needed genetic variations. There have been many efforts to identify wild sources of *Cicer* resistant to biotic stresses such as *C. judaicum*, *C. montbrettii* and *C. pinnatifidum* resistant to Ascochyta blight; *C. judaicum* resistant to Fusarium wilt; *C. pinnatifidum* and *C. judaicum* resistant to grey mold and *C. bijugum* resistant to cyst nematode (Singh *et al*., 1993). ICARDA (2000a) has identified important wild species like *C. bijugum*, *C. judaicum* and *C. pinnatifidum* possessing multiple stress resistance. *C. echinospermum* has seeds of a similar size to those of cultivated chickpea and is resistant to bruchids, leaf miner and AB (Singh *et al*., 1991). However, to the best of our knowledge there are no reports available so far

Table 1: Crossability groups of chickpea

Species	Crossability group
C. arietinum, C. reticulatum ,C. echinospermum	Group I
C. bijigum, C. pinnatifidum, C. yamashitae, C. judaicum	Group II
C. chorassanicum	Group III
C. cuneatum	Group IV

for the successful introgression of these genes in *C. arietinum*. Among the wild species of *Cicer*, *C. reticulatum* is cross compatible with chickpea (*C. arietinum*), whereas crossability of *C. echinospermum* with chickpea is low resulting in sterile F_1 hybrids (Ladizinsky and Adler, 1976; Pundir and Van der Maesen, 1983). Singh and Ocampo (1993) also found numerous transgressive segregants for high yield in F_2 populations. The performance of these lines suggested that genetic reshuffling, originating from interspecific hybridization, could produce favorable combinations of genes expressing high yield. Based on genetic relationships, crossability and fertility of hybrids in interspecific crosses, Ladizinsky and Adler (1976) have divided nine annual species of *Cicer* into four groups (Table 1).

Later on, Ahmad *et al.* (1988) have investigated the barrier to interspecific hybridization within and between the crossability groups of chickpea. This study revealed that, species in the primary, secondary and tertiary gene pools of chickpea are determined as follows where in most of the species defined in the second, third and fourth crossability groups, except *C. cuneatum* and *C. yamashitae* defined by Ladizinsky and Adler (1976) formed the tertiary gene pool.

- **Primary gene pool** : *C.arietinum, C. reticulatum, C. echinospermum*
- **Secondary gene pool** : No species
- **Tertiary gene pool** : *C. bijigum, C. pinnatifidum, C. judaicum, C. chorassanicum, C. montbretii*

Intraspecific hybridization: During last decades, ICRISAT has evaluated more than 12,000 accessions and identified several hundreds of resistant to AB (ICRISAT, 2003). ICARDA also bred more than 1600 lines resistant to AB and shared with national programs releasing ultimately, 39 cultivars in 12 countries (ICARDA, 2000b).

Diallel mating designs have been attempted in chickpea to obtain segregants giving high yields and resistance to diseases. The inheritance of ascochyta blight resistance and different leaf types and their correlation were investigated in intraspecific progeny derived from crosses among two resistant genotypes with normal leaf type (ICC 3996 and Almaz), one susceptible simple leaf type (Kimberley Large) and one susceptible multipinnate leaf type (24 B-Isoline) (Aryamanesh, 2007). Susceptibility to ascochyta blight was not correlated to multipinnate or simple leaf types in these segregating populations. Ascochyta blight resistance depends more on the genetic background than leaf type.

Screening technique: An easy and reliable screening technique for the field evaluation of large number of germplasm and breeding lines was developed at ICARDA (Singh *et al.*, 1981). In brief, it comprises (1) planting susceptible chickpea at frequent interval and all around the nursery plot, (2) inoculating the nursery with diseased debris collected in the previous season, (3) providing sprinkler irrigation to raise the relative humidity and (4) re-inoculating the nursery with spore suspension prepared in the laboratory for uniform development and spread of disease.

Disease assessment: Two methods were used to assess disease severity 14 days after inoculation. The first method was based on the 1-9 rating scale, which was modified for seedling bioassays from Reddy and Singh (1984), as follows: 1, healthy plant, no disease; 2, lesions present, but small and inconspicuous; 3, lesions easily seen, but plant is mostly green; 4, severe lesions clearly visible; 5, lesions girdle stems, most leaves show lesions; 6, plant collapsing, tips die back; 7, plant dying, but at least three green leaves present; 8, nearly dead plant (virtually no green leaves left) but still with a green stem and 9, dead plant (almost no green parts visible). In the second method, the number of leaves showing symptoms or wilting and the total number of leaves on each plant were counted and the percentage of infected leaves for each plant calculated (Kanouni *et al.*, 2010). This leaf counting method provided a more objective and quantitative estimate of disease (Chen *et al.*, 2004).

Inheritance of resistance: Complete resistance to Ascochyta blight has not been found in chickpea. Several authors acknowledge that known resistance sources show low degrees of infection but none show no infection at all, thus describing incomplete resistance (Lichtenzveig *et al.*, 2002). Hence, chickpea breeding programs rely mainly on genotypes with incomplete resistance, in which some symptoms, though of lower severity, are observed. Complete resistance is usually based on a discrete qualitative response, whereas incomplete resistance regularly shows quantitative continuum and therefore should be assessed using a quantitative scale. Ahmad *et al.* (1952) reported that disease resistance was controlled by two dominant complimentary genes. In desi cultivars, the resistance was governed by a single dominant gene (Taleei *et al.*, 2009b; Vir *et al.*, 1975; Eser, 1976; Hafiz and Ashraf, 1953). In *kabuli*, resistance to AB was governed by a single recessive gene for one cultivar and one dominant gene in several cultivars (Singh and Reddy, 1983). Dey and Singh (1993) reported that two dominant complimentary genes were responsible for resistance in two chickpea genotypes and one dominant and one recessive gene were controlling resistance in another. Tewari and Pandey (1986) reported that AB resistance in chickpea was governed by two recessive genes through additive gene action. According to Kusmenoglu (1990), resistance to ascochyta in chickpea was regulated by two recessive genes. Tekeoglu *et al.* (2000) reported that resistance was controlled by two quantitatively inherited major complimentary recessive genes and other minor genes.

Diversity and phylogeny of chickpea as revealed by molecular marker studies: Genetic diversity studies in a crop are important for various aspects such as management of genetic resources, identification of duplicate accessions in the germplasm and in applied breeding programs. In initial studies low copy sequence RFLP markers revealed very low levels of polymorphism (Udupa *et al.*, 1998) while, microsatellite based RFLP markers later demonstrated the potential of detecting higher polymorphism in chickpea (Weising *et al.*, 1998; Sharma *et al.*, 2005). In their extensive studies, Serret *et al.* (1997) analyzed the genetic diversity among different accessions of cultivated chickpea of the Kabuli type from different countries of the Eastern and Central Asia and Mediterranean regions using the (GATA)n probe. The genetic distance data revealed that the genetic diversity in chickpea was the greatest in Pakistan, Iraq, Afghanistan, South-East Russia, Turkey and Lebanon and lower in Iran, India, Syria, Jordan and Palestine. It was further concluded that there are three centers of diversity for chickpea namely Pakistan-Afghanistan, Iraq-Turkey and Lebanon.

Genetic linkage map of *Cicer* genome: The first rudimentary gene map in chickpea was reported by Gaur and Slinkard (1990) which included 26 isozyme and 3 morphological loci. Kazan *et al.* (1993) examined the linkage relationships of the genes for several isozymes and morphological traits and extended the linkage map of chickpea on the basis of F_2 population derived from the crosses between two cultivated lines and between cultivated line and the wild species *C. reticulatum* and *C. echinospermum*. Advances in molecular marker technology have accelerated the progress of genome mapping in chickpea. Linkage maps have been developed based on interspecific crosses between selected chickpea cultivars and *C. reticulatum* accessions (Banerjee *et al.*, 2001; Kazan *et al.*, 1993; Simon and Muehlbauer, 1997; Winter *et al.*, 1999, 2000). The first molecular map of chickpea using STMS markers was reported by Winter *et al.* (1999). In this study, initially locus specific primer pairs were designed for 22 microsatellite containing regions from *C. arietinum* which also generated amplification products in *C. reticulatum*, the closest relative of cultigens. Characterization of the simple sequence repeats from *C. arietinum* genome and amplification of the alleles in the wild progenitor made them a favorite choice for linkage map construction of the *Cicer* genome. The map generated using these STMS markers covered 613 cM, whereas the previous chickpea map covered 550 cM (Simon and Muehlbauer, 1997).

Initial efforts of tagging AB resistance genes in chickpea: Marker assisted selection can be applied effectively using DNA markers linked to resistance genes for accelerating disease resistance breeding programs. Such a phenomenon may be the result of gene duplication, exon shuffling and recombination processes that are thought to have generated different resistance genes from one or a few ancestral progenitor genes (Michelmore, 1995; Hammond-Kosack and Jones, 1997).

QTL mapping for Ascochyta blight resistance

Current status: The genetics of resistance to AB has been extensively analyzed because the disease is of great agronomic and economic importance. However, the emerging picture is confusing: depending on the fungal isolate and the cultivar, either one dominant, one recessive and one dominant, or one recessive resistance gene was reported. Also, two complementary recessive, or two complementary dominant genes were detected. At present, it is not clear whether the reported resistance genes represent the same or different loci because allelic tests were not performed (Winter *et al.*, 2000). To complicate the picture even more, other genes may modify the expression of resistance. Another bottleneck for mapping of ascochyta resistance genes is that, in many chickpea growing areas, several patho- and genotypes of the fungus may coexist in the same field or even in the same lesion (Peever *et al.*, 2004). Since, random mating may occur between different pathotypes of the fungus carrying different mating type alleles (Barve *et al.*, 2003), genetic recombination may contribute to genotypic diversity and provide the fungus with an additional means to adapt to newly introduced resistant germplasm (Peever *et al.*, 2004).

Different methods are applied for assessment of disease severity. Testing under controlled glasshouse or growth chamber conditions (Singh *et al.*, 1992; Udupa and Baum, 2003) combined with field screening (Flandez-Galvez *et al.*, 2003a; Millan *et al.*, 2003; Cho *et al.*, 2004) would very much help to improve the reproducibility of the results, since, severity and spread of the disease are highly dependent on environmental conditions and especially on humidity (which may change from year to year). Indeed, Cho *et al.* (2004) observed dramatic increases in severity of blight symptoms, if 100% relative humidity was maintained for more than 2 days after inoculation, as compared to normal greenhouse conditions. Further, different loci may contribute to resistance at different

points of the life cycle of the plant (Collard *et al.*, 2003). As the scale used for disease evaluation to blight (1-9 scale; Singh and Saxena, 1999; Reddy and Singh, 1984) is subjective particularly for intermediate values, a bias may be introduced by the researcher that may also affect the accuracy of tagging blight-resistant genes with markers. In fact, some authors detected different QTLs measuring disease reaction in the same environment using two disease scoring systems (Flandez-Galvez *et al.*, 2003a).

Ascochyta blight resistance is considered as a quantitative trait. In a study for identifying molecular markers for AB resistance, recombinant inbred lines from an interspecific cross between *C. arietinum* and *C. reticulatum* were subjected to marker analysis and molecular markers linked to two major QTLs, QTL1 and QTL2 were identified which together accounted for 50.3% of the estimated phenotypic variation, (Santra *et al.*, 2000).

Santra *et al.* (2000) identified two quantitative trait loci (QTL), QTL1 and QTL 2 conferring resistance to ascochyta which together accounted for 50 and 45% of variation in blight reaction over two years, respectively. Flandez- Galvez *et al.* (2003b) reported that resistance to AB under both field and controlled environments was associated with the genomic regions on LG1, LG2 and LG3. However, it was later found that the major QTL for AB resistance was located on LG4 (Udupa and Baum, 2003; Taran *et al.*, 2007). Taran *et al.* (2007) also reported one QTL on each of LG3 (16%), LG4 (29%) and LG6 (12%). The QTL on LG3 region was unique to the population derived from a cross involving ICCV96029 and CDC Frontier. Linkage group 4 (LG4) has a significant effect on the ascochyta blight resistance in chickpea whereas other genomic regions have minor effects (Table 2, Fig. 6).

Aryamanesh *et al.* (2010) found three quantitative trait loci (QTLs) explaining approximately 49% of the phenotypic variation for ascochyta blight resistance on LG3 and LG4. AB resistance was negatively correlated with flowering time, but not correlated with plant growth habit.

Three QTLs for resistance to ascochyta blight were found in the mapping population of Kanouni *et al.* (2009). The first one, located on linkage group 3 (LG3) (flanked by markers TA 34 and TA 142), explained 22.7% of the phenotypic variation for ascochyta blight resistance. Two linked QTLs, 12.9 cM apart on LG4, each explained 21.1 and 4.9% of the phenotypic variation suggesting that linkage group 4 has a significant effect on ascochyta blight resistance. These three QTLs explained 48.8% of the phenotypic variation in the population suggesting the involvement of several minor genes or robust effects of the environment on the trait. The significance of LG4 in containing QTLs for ascochyta blight resistance has been reported by several researchers (Cho *et al.*, 2004; Santra *et al.*, 2000; Tekeoglu *et al.*, 2002; Taran *et al.*, 2007). Unlike previous reports, they did not detect any QTL on LG2 (Cho *et al.*, 2004; Udupa and Baum, 2003) or LG8 (Lichtenzveig *et al.*, 2006). These findings were particular pertinent considering that they used Ascochyta rabiei pathotype III and ICC 12004 (resistant to pathotype III) for the first time (Taleei *et al.*, 2008, 2009a).

There are some factors that affecting the detection of QTLs segregating in a population (Asins, 2002). The main ones are genetic properties of QTLs that control traits, environmental effects, population size and experimental error. According to different studies, RIL or DH populations are ideal for QTL analysis. The larger the population, the more accurate the mapping study and the more likely it is to allow detection of QTLs with smaller effects (Haley and Anderson, 1997). An increase in population size provides benefits in statistical power, estimate of gene effects and confidence intervals of the locations of QTLs (Beavis, 1998; Collard *et al.*, 2003). A reliable QTL map can only be produced from reliable and accurate phenotypic data. Replicated phenotypic

Table 2: QTLs for resistance to ascochyta blight and related markers allowing their assignment to Linkage Groups (LG) of the map of Winter *et al.* (2000), updated from (Aryamanesh, 2007)

Pathotype	Name of QTL	Indicative markera	LG	References
nd	1	GAA47	4	Tekeoglu *et al.* (2002)
nd	2	TA72, GA2	4	
nd	1	TS12b		Flandez-Galvez *et al.* (2003b)
	2/3	TA3a/TA3b		
	4/5/6	TA30/TA146/TR20		
nd	AR2	SC/OPK13$_{603}$	4	Millan *et al.* (2003)
		SC/OPM02$_{935}$		Iruela *et al.* (2006)
		TA72, TA146		
nd	I	STMS11, GA2, GAA47, TR20	4	Rakshit *et al.* (2003)
I	ar1	GA16	2	Udupa and Baum (2003)
II	ar2a	GA16	2	
II	ar2b	TA130, TA72, TS72	4	
I	ar1a	GA20, GA16	2B-6B	Cho *et al.* (2004)
II	ar1b	TA37, TA200	2B	
II	ar2a	GA24, GAA47	4A	
nd	1*	STMS 11, GA2, TR20	4	Collard *et al.* (2003)
nd	2*	XLRRb$_{280}$	4	
nd	QTL4.1	H3C041, TA2	4	Lichtenzveig *et al.* (2006)
	QTL4.2	H1A12/H1H13, H1G20	4	
	QTL8	H1C092, TA3/H3C11a	8	
II	1	TA64, TS19	3	Taran *et al.* (2007)
	2	TA2, TA146	4	
	3	TA80, TA22	6	
II	QTL3[9]	TA34, TA142	3	Aryamanesh (2007)
	QTL4[1]	STMS11, TAA170	4	
	QTL4[7]	H3D09, H1A12	4	
III	1	TA125,TA34	3	Kanouni *et al.* (2009)
	2	TA2,TA72	4	
	3	GA26,TA80	6	

measurements or the use of clones (via cuttings) can be used to improve the accuracy of QTL mapping by reducing background noise (Haley and Anderson, 1997; Aryamanesh *et al.*, 2010). Some studies include those where phenotypic evaluations have been conducted in both field and glasshouse trials, for ascochyta blight resistance in chickpea (Flandez-Galvez *et al.*, 2003a).

Future prospects: Progress has been done in chickpea research using several biotechnological tools in the last 10 years. Chickpea scientists have certainly benefited from the progress achieved in the model legumes, *Medicago truncatula* and *Lotus japonicus*, or better investigated crops like soybean or pea (Millan *et al.*, 2006). However, research in cereals like wheat and rice has been accelerated due to the integration of conventional and modern approaches. Similar progress needs to be achieved in chickpea, where several genetic resources among the wild germplasm are unexploited (Rakshit *et al.*, 2003). It is necessary to have a high-density chickpea genome map to identify molecular markers linked to important agronomical traits. These markers can assist in breeding programs for generating tailor made varieties complementary to the region-wise

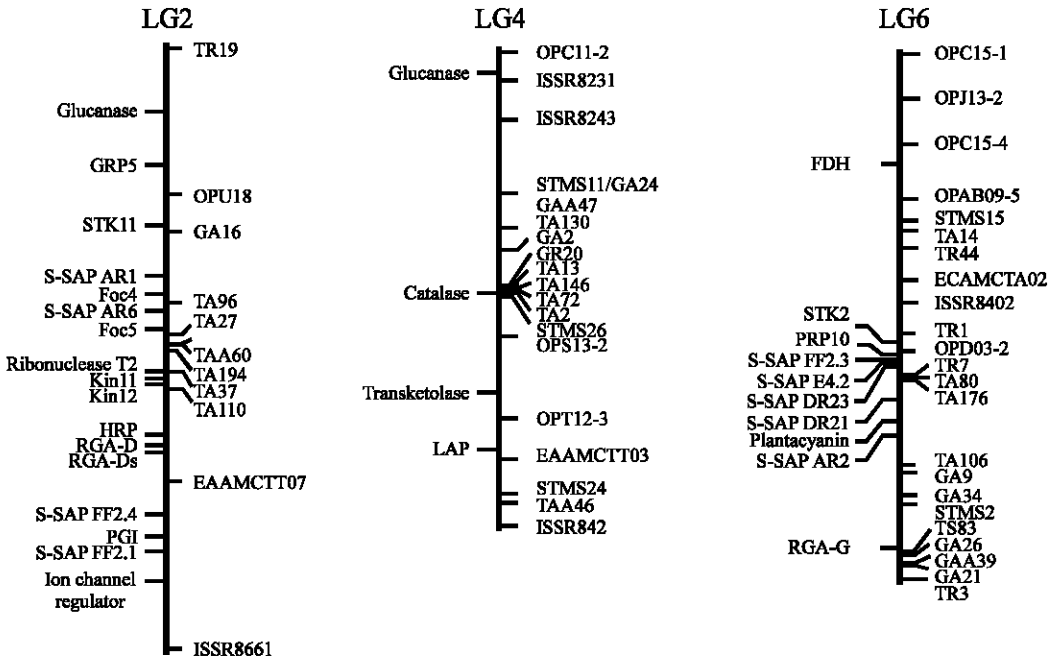

Fig. 6: Integrated genetic map for LGs 2, 4 and 6, most agreed linkage groups for QTLs conferring ascochyta blight resistance in chickpea. This map has constructed based on 131 RILs derived from the cross between ICC 4958 and *C. reticulatum* P.I. 489777, summarizing data from Winter *et al.* (2000), Huttel *et al.* (2002) and Pfaff and Kahl (2003). Markers on the left of the vertical bar are derived from genes, those on the right are STMS or dominant framework markers. Of the available markers, only a few necessary for demonstrating the context within the linkage groups are shown

requirements. Further, large insert BAC libraries of any crop are useful for positional cloning and identification of important genes (Rajesh *et al.*, 2008).

Genomic *in situ* hybridisation (GISH) is the most effective and precise technique for estimating the amount of foreign chromatin in translocation chromosomes (Gupta and Roy, 2002). Therefore, GISH should be used to investigate the post-zygotic barriers involved in crosses between chickpea with incompatible *Cicer* species and to also understand the cause of distortion of molecular markers and traits towards wild parents in segregating populations of interspecific hybrids.

Cicer bijugum is a species with several major resistance genes to ascochyta blight; therefore, cross between cultigen and this species need to be done using embryo rescue and other cytogenetic approaches (Aryamanesh, 2007). Findings of different researches suggest that wild *Cicer* species are needed to create mapping populations even though they do not possess resistant genes (Cobos *et al.*, 2005). This will help to understand future problems arising from interspecific hybridization.

The future will certainly see much more impact of transcriptomics in chickpea breeding including application of microarrays (Coram *et al.*, 2007). Thus, it will soon be possible to identify genes controlling complex traits by simply hybridising cDNAs to specialised chips. If combined with maps generated from markers detecting.

SNPs in differentially expressed genes such as single nucleotide amplification polymorphism markers (SNAP; Drenkard *et al.*, 2000; Hayashi *et al.*, 2004), it may be possible to land directly in

the gene of interest as demonstrated for mouse (Schadt *et al.*, 2003). The determination of genetic variability in these genes in the chickpea germplasm will then be the next step towards targeted molecular breeding and more efficient germplasm management.

The latest trends are to combine QTL mapping with functional genomics to study of gene expression. These techniques include Expressed Sequence Tag (EST) and microarray analysis, which can be utilized to develop markers from genes themselves. The use of gene sequences derived from ESTs or gene analogues, described as the 'candidate gene approach,' holds much promise in identifying the actual genes that control the desired traits. These methods can also be utilized to identify SNPs. EST-derived and SNP markers are usually integrated into existing maps that have already determined the locations of QTLs. Furthermore, the number of EST and genomic sequences available in databases is growing rapidly (especially from genome sequencing projects e.g. rice) and the accumulation of these sequences will be extremely useful for the discovery of SNPs and data mining for new markers in the future

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